REMARKS

Claims 1 and 5-24 are pending. Claims 21-23 are withdrawn from consideration. Claims 5, 5-20 and 24 are rejected.

Amendments to the Specification

Applicants have amended the Title of the present application to more accurately reflect the subject matter of the pending claims. The amendment is supported by the specification. The amendment does not introduce new matter. The Examiner is respectfully requested to enter the amendment.

Amendments to the Claims

Applicants have amended withdrawn claims 21 and 23. The amended claims are supported by the specification. The amendments to the claims do not introduce new matter. The Examiner is respectfully requested to enter the amendments to the claims.

Claim Rejections – 35 U.S.C. § 103

Claims 1-9 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Burns et al. (U.S. Patent No. 5,284,133) for the reasons of record set forth on page 7-10 of the Office Action mailed June 2, 2006 (the "June 2, 2006 Office Action"). Office Action mailed January 8, 2008 (the "January 8, 2008 Office Action") at 4.

In the June 2, 2006 Office Action, the Examiner stated that Burns et al. teaches that many drugs, including analgesics, could advantageously be delivered by aerosol inhalation. The Examiner also interpreted a passage in Burns et al. as teaching that loxapine hydrochloride was a known headache analgesic. (June 2, 2006 Office Action at 8, lines 19-20). The Examiner concluded that "[i]t would have been apparent to a person of ordinary skill in the art at the time of the instant invention that one could utilize Burn's inhalation device to deliver loxapine hydrochloride in the practice of a method of treating pain, because [Burns et al. teaches] loxapine hydrochloride is a known headache analgesic" (*Id.* at 9, lines 11-14).

However, the passage cited by the Examiner does not teach that loxapine hydrochloride is a known headache analgesic. The passage merely lists a variety of drug classes ("neuroleptics, psychotropics, narcotic antagonists, other central nervous system (CNS) drugs and headache analgesics") and then lists variety of drugs ("such as proclorperazine, fluphenazine hydrochloride, chlorpromazine, trifluperazine hydrochloride, thioridazine hydrochloride, loxapine hydrochloride, and haloperidol decanoate") as part of a long sentence concerning drugs for which there may be a tendency of some patients to overdose themselves. Burns et al., col. 7, lines 12-28.

Applicant submits that the passage may be fairly interpreted as teaching the listed drugs belong to one, or perhaps more than one, of the listed classes. However, it is inappropriate either to interpret this passage to mean that *each* of the listed drugs belongs to *each* of the listed drug classes, or to select one of the listed drugs and assign it to one of the listed categories. The Examiner's interpretation is not how a person of ordinary skill in the art would have understood the meaning of the passage. The Drug Information Handbook, 2nd edition, cited by the Examiner, for example, refers to loxapine's "onset of neuroleptic effect" (Drug Information Handbook, at 555; emphasis added). Applicant notes that "neuroleptics" is the first drug class listed in the passage cited by the Examiner that includes loxapine hydrochloride.

Applicants submit that the selection of the drug "loxapine hydrochloride" from among the various listed drugs, and assigning it to the class "headache analgesics" from among the various listed classes, was based not on the teachings of Burns et al, but rather on the teaching of Applicant's specification. It is impermissible, however, to engage in a hindsight reconstruction of the claimed invention using Applicant's specification as a template and selecting elements from a reference.

In response to Applicants' argument that it is inappropriate to reach the teachings of Burns et al. to mean that loxapine hydrochloride was a known headache analgesic, the Examiner states that "this argument was previously rebutted in the office action mailed on 2/15/2007 and the Office's position is unchanged." January 8, 2008 Office Action at 5. However, the Office Action mailed on February 15, 2007 (the "February 15, 2007 Office Action") does not address Applicants' argument, but provides only the conclusory

statement that "[t]he Examiner respectfully disagrees that Burns does not teach the utility of loxapine hydrochloride as a headache analgesic and that the interpretation of the teachings of Burns set forth in the office action mailed on June 2, 2006 is incorrect." February 15, 2007 Office Action at 6.

In response to Applicant's argument that the Examiner's conclusion of obviousness is based on improper hindsight reasoning, the Examiner cites *In re McLaughlin*, 443 F.2d 1392, 1395, 170 USPQ 209 (CCPA 1971) for the proposition that "any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper." January 8, 2008 Office Action at 5 (emphasis added). As indicated above, Applicants submit that the Examiner's reconstruction is of the latter, improper type because it is based on knowledge gleaned from Applicants' disclosure, rather than knowledge which was within the level of ordinary skill at the time the claimed invention was made.

Claims 5 and 9 further require that the headache is a "migraine headache." Significantly, later in the same sentence cited by the Examiner, Burns et al. excludes loxapine hydrochloride from the list of drugs in the class of "migraine headache analgesics" (Burns at col. 7, lines 25-27). Thus, if anything, Burns et al. should be interpreted as teaching that loxapine hydrochloride is not a drug for the treatment of migraine headache. Thus, for at least this additional reason, claims 5 and 9 are not obvious over Burns et al. because Burns et al. teaches away from using loxapine hydrochloride for the treatment of migraine headache.

In response to this argument, the Examiner states that "the Burns list is clearly not intended as being an exhaustive list of every known migraine analgesic and the mere fact that Burns chose not to explicitly cite loxapine hydrochloride as a migraine analgesic does not lead one to conclude that loxapine hydrochloride is not a migraine analgesic, nor would the teachings of Burns in column 7, lines 25-27 discourage an ordinary skilled

artisan from using loxapine hydrochloride to treat a migraine." January 08, 2008 Office Action at 5. Applicants respectfully disagree. The fact that Burns et al. place loxapine hydrochloride under the drug classification of "neuroleptics, psychotropics, narcotic antagonists, other central nervous system (CNS) drugs and headache analgesics" rather than the drug classification "migraine headache analgesics" undercuts the interpretation of the passage that is urged by the Examiner.

Burns et al. fails to teach or suggest the use of loxapine hydrochloride in the treatment of headache, or migraine headache, as claimed by Applicant. Thus, the Examiner has failed to establish a *prima facie* case of obviousness, as each and every element of claims 1-9 and 24 is not taught or disclosed by Burns et al.

Claims 10-15 are rejected under 35 U.S.C. § 103(a)as being unpatentable over Burns et al. as applied to claims 1-9 and 24, and further in view of the Drug Information Handbook, 2nd edition for the reasons of record set forth on page 11-12 of the June 2, 2006 Office Action.

The Examiner acknowledges that Burns et al. lacks teaching of loxapine dosages, but asserts that these are supplied by the Drug Information Handbook. As discussed above, Burns et al. fails to disclose the use of loxapine for the treatment of headache. The Drug Information Handbook, does not cure this deficiency. In fact, the Drug Information Handbook, indicates that loxapine is used for the treatment of psychotic disorders, giving no indication that the drug could be used in the treatment of headache. Drug Information Handbook at 554.

Burns et al. in view of the Drug Information Handbook fails to teach or suggest the use of loxapine in the treatment of headache. Thus, for at least this reason, the Examiner has failed to establish a *prima facie* case of obviousness, as each and every element of claims 10-15 is not taught or disclosed by Burns et al. in view of the Drug Information Handbook.

Moreover, the dosages taught by the Drug Information Handbook are for the treatment of psychotic disorders. *See, e.g.*, Drug Information Handbook at 555 ("10 mg twice daily, increase dosage until psychotic symptoms are controlled") (emphasis added).

These dosages taught by Drug Information Handbook to treat psychotic symptoms would not convey to one of skill in the art what dosages are appropriate to treat headache. See specification at paragraphs [0024]-[0025].

Thus, at least for this additional reason, claims 10-15 are not obvious over Burns et al. in view of the Drug Information Handbook.

In response to Applicants' argument, the Examiner states that "it would have been apparent to a skilled artisan that the dosages required for inhalation administration would be lower than those for oral administration (DIH), because via inhalation administration the disadvantage of first-pass metabolism of the administered drug by the liver and kidneys is avoided (Burns). Therefore a lower amount of drug would be needed if administered by inhalation. The skilled artisan would utilize the teachings of the DIH regarding the oral doses as a maximum starting point from which to undertake routine optimization of the dosage amounts as practiced in the art and it would have been well within the skill of the ordinary skilled artisan to ascertain what dosage amount is suitable for effective analgesia of headaches and migraines." January 08, 2008 Office Action at 6. However, the Examiner's response does not address Applicants' argument that dosages taught by the DIH are for the treatment psychotic disorders. Without some reason for doing so, one of skill in the art would not assume that the dosage of a drug required for one indication would be the same for another indication. The Examiner has not provided any such reason. Moreover, the DIH indicates that an initial dose of 10 mg be administered twice daily and that the dosage be increased until psychotic symptoms are controlled. It is not clear how one of skill in the art would take this instruction to "increase dosage until psychotic symptoms are controlled" as a starting point to arrive at a dose that is effective in treating headaches. Applicants teach at paragraphs [0024]-[0025] how the dosage of loxapine to treat migraine headache differs from the dosage of loxapine for treatment of schizophrenia.

Claims 16-17 and 19-20 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Burns et al. as applied to claims 1-15 and 24, and further in view of

Nguyen et al. (U.S. Patent No. 7,040,314) for the reasons of record set forth on page 12-15 of the Office Action mailed June 2, 2006.

As discussed above, Burns et al. fails to disclose the use of loxapine for the treatment of headache. Nguyen does not overcome this deficiency. Nguyen discloses loxapine in its known role as an anxiolytic, but not in the treatment of headache.

Burns et al. in view of Nguyen fails to teach or suggest the use of loxapine in the treatment of headache. Thus, for at least this reason, the Examiner has failed to establish a *prima facie* case of obviousness, as each and every element of claims 16-17 and 19-20 is not taught or disclosed by Burns et al. in view of Nguyen.

Claims 16-18 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Burns et al., in view of Rabinowitz et al. (US 2004/0009128) for the reasons of record set forth on page 15-17 of the Office Action mailed June 2, 2006.

As discussed above, Burns et al. fails to disclose the use of loxapine for the treatment of headache. Because Rabinowitz et al. does not overcome this deficiency, the Examiner has failed to establish a *prima facie* case of obviousness, as each and every element of claims 16-18 is not taught or disclosed by Burns et al. in view of Rabinowitz et al.

In response to Applicants' argument that the secondary references Nguyen and Rabinowitz et al. do not cure the deficiencies of Burns et al., the Examiner states that the argument "is not material" because Applicants' argument that Burns et al. failed to disclose the administration of loxapine hydrochloride to treat headache "has been clearly and unambiguously rebutted." January 8, 2008 Office Action at 7. However, as mentioned earlier, the Office Action mailed on 2/15/2007 (the "February 15, 2007 Office Action") does not address Applicants' argument, but provides only the conclusory statement that "[t]he Examiner respectfully disagrees that Burns does not teach the utility of loxapine hydrochloride as a headache analgesic and that the interpretation of the teachings of Burns set forth in the office action mailed on June 2, 2006 is incorrect." February 15, 2007 Office Action at 6. Moreover, claims 16-20 are not rejected over

Burns et al. alone, they are rejected over Burns et al. in view of either Nguyen et al. or Rabinowitz et al. Thus, Applicants' arguments are material because if Nguyen et al. and Rabinowitz et al. do not cure the deficiencies of Burns et al, then claims 16-20 are patentable.

The Examiner has rejected claims 1 and 10-15 under 35 U.S.C. § 103(a) as being unpatentable over Dehaven et al. (WO 02/060870). January 8, 2008 Office Action at 8-9.

The Examiner states that Dehaven et al. teaches methods of inducing analgesia in a patient comprising administration of compounds of formulas (I) and (Ib), both of which encompass loxapine. The Examiner acknowledges that Dehaven et al. does not state that the compounds of formula (I) or (Ib) are intended for headache or migraine. However, the Examiner contends that it would have been obvious at the time of the instant application to administer loxapine to treat the pain associated with a headache and that an ordinary skilled artisan would have had a reasonable expectation of success of treating a headache upon administration of loxapine because analgesics are conventionally administered to treat pain, headaches are characterized by the sensation of pain, and loxapine has analgesic properties.

However, as Applicants have argued before, Dehaven et al. uses loxapine as a negative control in comparison to the stated preferred embodiments of N-desmethylclozapine and amoxapine and that Dehaven et al. teaches away from using loxapine as an analgesic because loxapine does not have the requisite delta opioid receptor agonist activity. *See* Amendment and Remarks mailed November 30, 2006 at page 7-8, incorporated herein by reference.

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The data in Dehaven's Table 1 do not support an analgesic activity for loxapine. The data show that loxapine binds to the delta opioid receptor very weakly, with 10 μM loxapine resulting in only 26% inhibition of dinprenorphine binding (notably, binding to the kappa opioid receptor is similarly weak and to the mu opioid receptor unmeasurable). The weak binding of loxapine is in contrast to the strong binding of N-desmethylclozapine (DMCLZ), which produces 50% inhibition at 0.024 μM. Thus,

loxapine is at least 400-fold less potent in binding to the delta opioid receptor than DMCLZ. In light of this low potency, which suggested that loxapine would not have *in vivo* analgesic properties, Dehaven did not test the *in vivo* analgesic activity of loxapine. However, Dehaven did test the analgesic activity of DMCLZ, which resulted in 50% inhibition of acetic-acid induced writhing in mice at a dose of ~ 3 mg/kg s.c. By extrapolation of the *in vitro* opioid-receptor binding properties of DMCLZ and loxapine, one can compute the loxapine dose expected to produce 50% inhibition of acetic-acid induced writhing:

$$EC_{50}^{loxapine} = EC_{50}^{DMCLZ} \times IC_{50}^{loxapine} / IC_{50}^{DMCLZ}$$

where $EC_{50}^{loxapine}$ refers to the dose of loxapine anticipated to produce 50% inhibition of acetic-acid induced writhing in mice; EC_{50}^{DMCLZ} refers to the dose of DMCLZ found to produce 50% inhibition of acetic-acid induced writhing in mice (3 mg/kg); $IC_{50}^{loxapine}$ refers to the concentration of loxapine producing 50% blockade of the delta opioid receptor *in vitro* (> 10 μM); and IC_{50}^{DMCLZ} refers to the concentration of DMCLZ producing 50% blockade of the delta opioid receptor *in vitro* (0.024 μM). See Dehaven et al. at page 16, lines 23-24 ("Useful dosages of the compounds of the present invention can be determined by comparing their in vitro activity, and in vivo activity in animal models"). These calculations yield an anticipated $EC_{50}^{loxapine}$ of > 1200 mg/kg.

The LD_{50} of loxapine in mice is reported to be about 65 mg/kg s.c. *See* Mineshita T, Muraoka Y, Yahara I, Inuta T, Ishikawa M, Kawaguchi J, Okada T. Toxicity Tests of 2-Chloro-11-(4-methyl-1-piperazinyl)-dibenzo [*b,f*] [1,4] oxazepine (S-805) (I) Acute, Subacute and Chronic Toxicity of S-805. *Oyo Yakuri Pharmacometrics* 4:293-303 (1970) (53 mg/kg s.c. (male); 76 mg/kg s.c. (female)). Thus, the dose of loxapine suggested by Dehaven et al. to be required for analgesia is greater than 10-fold higher than the lethal dose of loxapine. Accordingly, Dehaven et al. does not teach use of loxapine as an analgesic, and quite to the contrary taught that efforts in this direction should result in fatalities prior to onset of analgesia.

Furthermore, Dehaven et al. at most provides compounds that have a high affinity for the delta opioid receptor and thus may have some analgesic utility as a consequence

(i.e., because the compound acts as a delta opioid receptor agonist). Thus, one of skill in the art would have no reasonable expectation that the compounds taught by Dehaven et al. would be effective to treat any condition in the absence of evidence that the condition is mediated by the delta opioid receptor. The Examiner has provided no such evidence with respect to headache or migraine.

Reconsideration is respectfully requested.

Double Patenting

Claims 1, 16-17 and 19 are rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 7, 9, 10, 12 and 13 of U.S. Patent No. 6,716,416.

Claims 1 and 16-19 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 12, 15, 16 and 18 of copending Application No. 10/633,876. Claims 1 and 16-20 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 1 and 7-9 of copending Application No. 10/633,877. Claims 1 and 5-15 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 1 and 15 of copending Application No. 10/719,763. Claims 1 and 5-15 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1 and 15 of copending Application No. 11/346,548.

Applicants hereby agree to file appropriate terminal disclaimers in this application with respect to subject matter ultimately found to be patentable.

Conclusion

Applicants appreciate the Examiner's careful and thorough review of the application. Applicants request the Examiner to allow the application. In the event the Examiner believes a telephonic discussion would expedite allowance or help to resolve

outstanding issues, prosecution of the application, then the Examiner is invited to call the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to Deposit Account No. 19-5117, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to Deposit Account No. 19-5117.

Respectfully submitted,

Date: July 8, 2008

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応用薬理 (1970) 4 (2) 293-303

論 文

2-Chloro-11-(4-methyl-1-piperazinyl)-dibenzo[b,f][1,4] oxazepine (S-805) の毒性試験 (第1報)

急性, 亜急性および慢性毒性試験

峰下 哲郎, 村岡 義博, 矢原 功, 鴻田 忠義
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Toxicity Tests of 2-Chloro-11-(4-methyl-1-piperazinyl)-dibenzo[b,f][1,4]oxazepine (S-805) (I)

Acute, Subacute and Chronic Toxicity of S-805

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Toxicity studies of 2-chloro-11-(4-methyl-1-piperazinyl)-dibenzo[b,f][1,4]oxazepine were performed in both sexes of the mouse and the rat. The studies consist of; 1) acute toxicity tests by a single dosage in mice and rats by the oral, subcutaneous, intraperitoneal and intravenous routes, 2) thirty days toxicity tests in mice and rats by the oral route at dose levels of 5,10,20 and 40 mg/kg/day for mice, and 0.5, 2, 8, 32 and 64 mg/kg/day for rats, 3) six months toxicity test in rats by the oral route at dose levels of 2.5, 12.5 and 62.5 mg/kg/day.

Sedation, ataxia, catalepsy, ptosis and respiratory depression were the main toxic signs which appeared in both mice and rats throughout the acute experiments. LD_{50} values by four routes of administration ranged from 22 to 76 mg/kg for mice and 18 to 381 mg/kg for rats. There were little differences in the values due the mode of administration in mice, but rats showed a large variation due to poor absorption of the drug by the subcutaneous route.

The animals showed hypoactivity, catalepsy and decrease of food intake with a dose-response relation in the continuous administration experiments. These changes caused growth retardation and subsequent decrease of organ weights.

Slight fat deposits in the hepatic cells at the central area of the lobules were found only in the animals which died during the treatment.

Spontaneous morphological changes in the kidney and the islets of the pancreas, which appeared in the aged males or the SD-JCL strain were apparently reduced in the drug treated groups in the six months experiment. It is unknown whether these findings were due to a primary effect of the drug or secondary to the decreased food intake.

(Received November 14, 1969)

緒 言

Neuroleptics (major tranquilizer) は化学的には reserpine 群, benzoquinolizine 群, phenothiazine 誘, butyrophenone 群に分けて考えられて来たが, 最近 dibenzoxazepine 群の neuroleptics としての作用が注目されて来ている (Stille *et al* 1955).

今回, 動物実験において鎮静反射抑制作用, 自発運動抑制作用, アポモルフィン拮抗作用, 及びカタレプシー作用が認められ (成川ら 1969), ヒトに対する新しい neuroleptics として期待される dibenzoxazepine 誘導体 S-805 のマウス, 及びラットにおける, 急性, 亜急性, 慢性毒性を検討したので報告する.

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実験材料および方法

1. 薬物

S-805 は FIG 1 に示す化学構造をもち，その性状は白色ないし黄色，無味無臭の結晶性物質で，水には不溶であるが，塩酸を含む大部分の有機溶媒に易溶で，融点は 105～112° (分解) である。

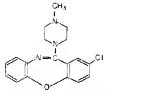

FIG 1 Chemical structure of S-805

2. 実験方法

全実験を通じて，ICR-JCL 系マウス，SD-JCL 系ラットを実験動物として使用した。動物は室温 24～26°，湿度 52～57% の動物室で維持し，油日ラボラトリーズ製固型飼料と水道水を与えた。

1) 急性毒性

薬物投与前 18～20 hr 絶食したマウス (20～29 g) およびラット (130～200 g) に薬物を 1 回量，皮下，腹腔内，静脈内に投与した。薬物は所定量を 1N HCl に溶解し，蒸留水ソーダで pH 4～4.5 として使用した。

マウス，ラット共に 1 用量当り 10 匹よりなる 4～9 用量を用い，観察期間は 3 日間とした。死亡例および 3 日目生存例は解剖し，肉眼的なよびに H-E 染色により顕微鏡的に観察した。

LD_{50} は Bliss 法 (Bliss 1938) により算出した。

2) 亜急性毒性

1 群 12 例よりなる 4 群のマウスおよび 5 群のラットに 1% アラビアゴムに懸濁した S-805 を 30 日間連続経口投与した。投与量はマウスに対して 1 日量 5, 10, 20, 40 mg/kg，ラットに対して 0.5, 2, 8, 32, 64 mg/kg とした。それぞれ 1 群は対照群とし，1% アラビアゴム液のみを投与した。投与期間中は一般状態，体重測定，死亡例の剖検，摂餌量の測定を行った。実験終了当各時より操作法に 10 例ずつとり出して血液検査，できうるかぎり解剖，臓器重量測定を行ない，マウスについてはそのうちの 7 例，ラットは 10 例ずつについて組織学的検査を行った。生存例が統計の判断に満たない群は全例について行った。

臓器重量は実験結果を参照，その他の検査方法の詳細は別報 (塚下他 1969) に譲った。

3) 慢性毒性

投与量は 1 日量 2.5, 12.5, 62.5 mg/kg とした。1 群の動物数は 10 例とし，対照群には 1% アラビアゴムのみを投与した。

投与期間は 6 カ月間 (26 週間) とした。

観察項目は別報 (塚下他 1969) の慢性試験に準じた。

実験結果

1) 急性毒性

中毒経過：マウス，ラット共にどの投与経路においても同様の中毒症状，自発運動の低下，筋弛，骨格筋の弱度強直，歩行失調，カタレプシーがみられ，マウスでは，死亡前に痙攣，強直，単居反応がみられた例があった。

発現時間は静脈内では投与直後，皮下および腹腔内では 10 min，経口では 20 min 以内であった。30 min 以後にはこれらの症状の他に呼吸，減弱，呼吸抑制，眼瞼下垂がみられた。これらの症状は 48 hr 後では殆ど消失するが，カタレプシーは持続が長く，72 hr 目 2 匹を非常となった。また，この時点では体重は増加し始める。動物の死亡は全て 24 hr 以内に終了する。LD_{50} 値は TABLE 1 に示す。

臓器の肉眼的および組織学的所見：死亡例ではマウス，ラット共に肝，肾，肺のうっ血がみられ，その他には著明な変化はなかった。3 日目のマウスの臓器例では，

TABLE 1 LD_{50} values of S-805 in mice and rats by single administration

Route	Mouse (mg/kg)		Rat (mg/kg)	
	Male	Female	Male	Female
po	67 (50～90)	62 (54～70)	221 (187～299)	151 (113～199)
sc	58 (44～82)	76 (70～82)	381 (331～439)	350 (292～409)
ip	34 (30～37)	34 (31～37)	35 (32～ 38)	37 (32～ 41)
iv	22 (20～24)	22 (20～24)	18 (15～ 19)	21 (19～ 23)

Figures in parentheses represent 95% confidence limits

経口投与例で軽度の肝の貧血がみられたのみであった．

ラットの死亡例では肝，腎，肺のうっ血，3日目屠殺例では肝，腎，脾，甲状腺に貧血がみられた．ラット皮下投与群では3日目屠殺例においても投与局所に少量の薬物が吸収されずに残っている例があった．

組織学的にはマウス，ラット共に著変はなかった．

2) 亜急性毒性

一般状態： マウスの薬物投与群では，全実験期間に亘って，薬物投与後 10 min 頃より動作の緩慢，軽度の筋弛緩，カタレプシーが観察され，症状の強さ，持続時間は投与量に比例した．ラットでは 0.5 mg/kg 群は実験初期にのみ，2, 8 mg/kg 群は中期にかけて，32, 64 mg/kg 群は全期間に亘ってカタレプシーおよび軽度の鎮静状態が観察された．

死亡例： 投与期間中の死亡例は TABLE 2 に示す通りであった．マウスの明らかに薬物による中毒死と思われる例は 40 mg/kg 群においてのみみられ，死亡時期は全例投与1週間以内であった．この群の死亡例では投与1日目より体重は減少し，強い鎮静状態，カタレプシー，流涎がみられた．剖検では肝，腎，肺のうっ血，脾の貧血と萎縮がみられた．これらの死亡例の組織学的所見では雄において肝細胞の萎縮と軽度の中心性脂肪沈着，雌では肝細胞の萎縮のみがみられた．

その他の臓器に著変はなかった．

ラットにおける死亡例は雄では 64 mg/kg 群にのみ，雌では 32 mg/kg 以上の群においてみられ，ラットではマウスと異なり雌において死亡例は多かった．死亡前には成長抑制がみられ，特に実験期間の前半に死亡した5例 (45%) で著明であった．死亡例の剖検所見では全例に肝，腎，肺のうっ血，半数例で脾の萎縮，少数例に鼻出血，胃の拡張がみられた．組織学的には前記の臓器のうっ血の他に肝細胞の中心性脂肪沈着が軽度にみられた．この変化は雌においてより強かった．

摂餌量および体重： マウスにおける体重抑制は雄では薬物投与全群に，雌では 40 mg/kg 群においてのみ認められ，それ以下の投与群では対照群以上の体重増加を示した．摂餌量は体重変化と同様の傾向を示し，雌 20 mg/kg 以下では飼料効率は対照よりむしろ良かった．ラットでは雄 2 mg/kg 以上，雌 32 mg/kg 以上の投与群で成長抑制があり，それに対応した摂餌量の減少がみられた (TABLE 2).

血液像： マウスの赤血球数には著変はなかった．白血球数は雄 10 mg/kg 以上の投与群において減少傾向がみられたが，雌では用量-作用関係のある変化はなかった．

ラットでは 64 mg/kg 群の雄において赤血球数，ヘモグロビン値，ヘマトクリット値の軽度増加があり，白血球数はこの群では雌雄共に増加した．白血球細胞型別百分率では薬物投与群において一般に好中球の増加，リンパ球の減少の傾向がみられた (TABLE 3).

臓器重量： マウスにおいて重量減少した臓器は肝 (雄, 10 mg/kg 以上)，胸腺 (雄, 40 mg/kg)，副腎 (雌, 10 mg/kg 以上) であり，重量増加した臓器は顎下腺 (雌, 20mg/kg 以上) であった (TABLE 4).

TABLE 2 Body weight and food consumption in mice and rats orally administered S-805 for 30 days

Species	Dose (mg/kg/day)	No. of animals		Average body weight gain (g)		Average food consumption (g/animal/30 days)		Food efficiency	
		Male	Female	Male	Female	Male	Female	Male	Female
Mouse	0[a)]	12(0)[b)]	12(1)	8.0	3.9	139	109	0.058	0.036
	5	12(1)	12(1)	4.6*	5.0*	142	107	0.032	0.047
	10	12(2)	12(0)	4.2*	5.0*	128	112	0.033	0.045
	20	12(1)	12(0)	3.7*	4.9*	127	129	0.029	0.038
	40	12(7)	12(4)	2.9*	3.3*	123	106	0.024	0.031
Rat	0[a)]	12(0)	12(0)	260.1	120.3	749	530	0.347	0.227
	0.5	12(0)	12(0)	242.1	124.8	700	514	0.346	0.243
	2	12(0)	12(0)	226.2*	118.6	678	492	0.334	0.241
	8	12(0)	12(0)	196.4*	106.1	602	460	0.326	0.231
	32	12(0)	12(3)	157.0*	69.7*	533	401	0.295	0.174
	64	12(2)	12(6)	82.7*	35.7*	393	349	0.210	0.102

a) 1% arabic gum.

b) Figures in parentheses indicate the number of animals that died during the 30 days of administration.

* Significant difference from controls, $p<0.05$.

TABLE 3 Hematological findings in mice and rats

Species	Dose (mg/kg/day)	No. of animals		Red blood cell ($\times 10^4$/cmm)		Hemoglobin (g/dl)	
		Male	Female	Male	Female	Male	Female
Mouse	0[a]	10	9	883	909		
	5	10	10	883	893		
	10	10	10	842	892		
	20	10	10	935*	852*		
	40	5	8	924	868		
Rat	0[a]	10	10	721	730	12.6	13.9
	0.5	10	10	7.7	706	13.5	14.0
	2	10	10	7.9	743	13.2	13.9
	8	10	10	732	705	13.0	13.8
	32	10	9	718	651[b]	13.1	13.4
	64	10	6	764*	770	14.4*	14.6

a) 1% arabic gum.

TABLE 4 Organ weight in mice orally administered

Sex	Dose (mg/kg/day)	No. of mice	Heart (mg)	Kidney (g)	Absolute Liver (g)
Male	0[a]	10	165	0.60	2.05
	5	10	150	0.57	1.84
	10	10	176	0.63	1.72*
	20	10	167	0.59	1.55*
	40	5	177	0.62	1.68*
Female	0[a]	9	124	0.33	1.28
	5	10	129	0.35	1.35
	10	10	124	0.32	1.23
	20	10	136	0.34	1.28
	40	8	123	0.35	1.29

a) Seminal vesicle for male, and ovary for female.
b) Testis for male, and uterus for female.

ラットの雄では 2mg/kg 以上，雌では 32mg/kg 以上の投与群において体重減少に対応した器官重量の減少がみられ，重量増加した臓器はなかった（TABLE 5）．

組織学的観察： 生存例のマウスでは肝臓に原因した変化は雄の肝においてのみみられ，雌では変化はなかった．肝の変化は軽度の肝細胞の腫脹と肝細胞質の軽度の好酸性の増加で，20, 40 mg/kg 群の約半数例に認められた．

ラットの生存例では軽度の肝細胞の腫脹（5/10 例）および肝細胞質の好酸塩基性顆粒の減少した例（3/10 例）が 64 mg/kg 群の雄にのみみられた．心筋の壁変増を示す例は 64mg/kg 群（雄 5/10 例，雌 2/6 例），32 mg/kg 群（雄 2/10 例，雌 1/10 例）に認められた．肺のリンパ濾胞周辺の細網細胞の増加を示す例，頸下腺終末部細胞の腫度腫脹を示す例が雄 64 mg/kg 群に散見された．

3) 慢性毒性

一般状態： 2.5mg/kg 群では実験初期，鎮静状態，カタンプシー様姿勢を示す例が約半数例にみられたが1カ月以上になると多黒群との差のない一般状態を示した．12.5mg/kg 以上の投与群では全例に上記の症状がみられ，特に 62.5mg/kg 群では上記の症状は強く，1週間以内に多数例が死亡した．

orally administered S-805 for 30 days

Hematocrit (%)		White blood cell (×1/mm³)		Differentiation (%) of WBC			
				Neutro.		Lymph.	
Male	Female	Male	Female	Male	Female	Male	Female
		6,040	5,060	17.7	18.0	79.8	77.6
		5,800	3,960*	24.2	20.6	72.9	75.9
		3,820*	5,150	24.0	16.2	73.8	81.2
		3,200*	3,580*	24.6	14.0	73.3	82.4
		3,850*	4,890	22.0	13.4	75.0	83.4
38.5	39.8	8,530	7,120	7.5	7.3	90.9	90.1
38.1	38.7	8,870	7,340	8.4	11.7*	88.4	84.8
37.9	39.9	10,000	9,040	10.6	14.5*	86.1	82.8*
37.5	39.0	7,800	9,170	15.3*	10.7	81.6*	87.1
37.7	34.7*	11,080	7,480	16.7*	15.0	80.4*	81.2
42.6*	39.2	12,220*	11,500*	11.4	12.7*	86.6	84.7*

* Significant difference from controls, $p<0.05$.

S-805 for 30 days

organ weight

Spleen (mg)	Adrenal (mg)	Thymus (mg)	Submax. gl. (mg)	Sex organ		Lung (mg)
				(mg)[a)]	(mg)[b)]	
144	5.5	36.5	196	138	235	245
197*	6.1	30.1	170*	159	231	295
149	5.8	35.9	179*	185	227	285
124	5.5	31.7	207	207	236	233
122	5.8	25.4*	224	136	232	264
127	9.7	48.5	106	10.9	119	190
146	9.3	48.7	128	16.4*	122	201
135	7.4*	45.2	112	13.6	134	217
167	7.6*	56.5	135*	14.9*	151	258
130	7.9*	51.5	131*	13.8	132	201

c) 1% arabic gum.

* Significant difference from controls, $p<0.05$.

死亡例： 死亡例の 64% は 1 週間以内にみられ，死亡前に一般に強い成長抑制を示した．これらの例の剖検では肺，肝，等のうっ血，消化管のガスを認めた拡張，脾の萎縮がみられた．組織学的には肺々，副急性気性試験の死亡例の所見に一致した．

体重および摂餌量： 雄では全投物投与群に用量 作用関係をもって雌では 62.5 mg/kg 群においてのみ成長抑制があり，それに対応した摂餌量の減少がみられた (TABLE 6, FIG 2).

血液像： 3 カ月目では 62.5 mg/kg 群において経度の赤血球数，ヘモグロビン値，ヘマトクリット値の減少傾向がみられるが，6 カ月目では対照群と差のない値となる．白血球数には 12.5, 62.5 mg/kg 群において 3 カ月目に経度に減少する．白血球細胞別百分率では雌において好中球の増加，リンパ球の減少がみられ，62.5 mg/kg 群では 6 カ月目においても認められた (TABLE 7).

臓器重量： 体重推移に比例して臓器重量は一般に減少した．個々の臓器により減少の程度はかなり異なるが雄の肝，腎，脾，心において著明である (TABLE 8).

血漿分析： 用量-作用関係のある変化に雌の S-GOT 値と総 cholesterol 値でとった．前者は 12.5, 62.5 mg/kg 群において高値が，後者は投物投与全群に低値

TABLE 5 Organ weight of rats orally administered

Sex	Dose (mg/kg/day)	No. of rats	Heart (g)	Kidney (g)	Liver (g)	Spleen (g)
			Absolute			
Male	0[a]	10	1.26	3.05	15.17	0.68
	0.5	10	1.19	2.85	14.54	0.93
	2	10	1.14	2.66*	13.42	0.79
	8	10	1.03*	2.36*	12.12*	0.76
	32	10	0.99*	2.44*	10.67*	0.70*
	64	10	0.79*	1.88*	8.80*	0.45*
Female	0[a]	10	0.77	1.75	8.16	0.62
	0.5	10	0.85	1.83	7.80	0.68
	2	10	0.80	1.66	8.23	0.59
	8	10	0.76	1.70	7.96	0.61
	32	9	0.65	1.57	7.56	0.49
	64	6	0.57*	1.43*	6.91*	0.42*

a) Ventral prostate for male, and uterus for female.
b) Testis for male, and ovary (mg) for female.

TABLE 6 Body weight and food consumption in rats orally administered S-805 for 6 months

Dose (mg/kg/day)	No. of rats		Average body weight gain (g)		Average food consumption (g/animals/26 w)		Food efficiency	
	Male	Female	Male	Female	Male	Female	Male	Female
0[a]	10(0)[b]	10(0)	569	285	4,199	2,902	0.136	0.098
2.5	10(0)	10(0)	473*	280	3,629	2,724	0.130	0.103
12.5	10(1)	10(1)	402*	246	3,283	2,641	0.122	0.093
62.5	10(7)	10(5)	302*	197*	2,813	2,430	0.107	0.081

a) 1% arabic gum.
b) Figures in parentheses indicate numbers of rats that died during 6 months of administration.
* Significant difference from controls, $p<0.05$.

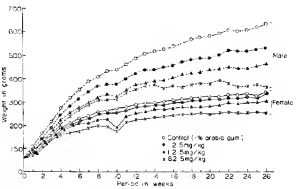

FIG 2 Growth curve of rats orally administered S-805 for 6 months.

S-805 for 30 days

organ weight

Adrenal (mg)	Thymus (mg)	Thyroid (mg)	Hypophysis (mg)	Submax. gl. (mg)	Sex organ (mg)a)	(g)b)	Lung (g)
46.8	610	16.6	12.3	550	376	3.03	1.57
42.5	660	21.2	9.8*	520	340	3.08	1.85
52.0	510	19.0	11.9	530	351	3.16	1.47
52.5	470*	21.7	11.3	450	290	2.91	1.72
47.2	460*	13.3	9.5*	480	231*	2.63	2.13*
47.1	460*	12.7*	8.4*	390*	195*	2.54	0.98*
57.3	458	12.5	11.5	392	268	73.8	1.03
54.4	481	14.3	11.1	403	333	74.9	1.29
54.8	456	12.2	9.1	401	247	68.4	1.02
52.5	403	13.5	11.0	392	281	70.5	1.14
54.4	287*	11.5	8.9*	323*	214	55.5*	0.95
48.8*	260*	12.6	6.4*	273*	248	48.3*	1.04

a) 1% arabic gum.
* Significant difference from controls, $p<0.05$.

があるわけ (TABLE 9).

組織学的所見： 雄では薬物による著明な障害は認められない．しかし，考合 SD JCL 系ラットにみられる特徴的な間質内細胞浸潤および管腔内にヒアリン円柱を満たし扁平化した上皮よりなる尿細管の拡張像，膵 Langerhans 島の線維化は薬物投与群では明らかに減少した．雌では肝細胞質の好塩基顆粒性の構造の減少を示す例が 12.5, 62.5 mg/kg 群にみられた．その他に薬物投与と関連した著しい変化はなかった (TABLE 10).

総　括

全実験を通じてマウス，ラットに共通した一般症状は等量栗に一般的なカタレプシーと鎮静作用であり，投与法，性に関係なくあらわれた．作用の強さ，持続時間は投与量に比例した．1回投与では致死量を投与しても死を免れた例は 72 hr 以内に正常となる．上記の作用は連続投与時の摂餌量の減少，成長抑制につながる作用と思われる．大量連続投与ではこの作用が高じて動物は1週間以内に衰弱するが，この期間を耐えた強大どの例は実験終了時まで生存した．

摂餌量の減少，成長抑制はマウス，ラット共に雄より雌に強く，雌マウス 20 mg/kg 以下の投与群ではむしろ対照群より大きな摂餌量，成長を示した．

血液像はマウス，ラット共に著明な変化はないが白血球数の減少が雄マウス，10 mg/kg 以上の投与群にみられた．ラットでは 64 mg/kg 群の雌雄に白血球数の増加が，雌でのみ赤血球数の軽度増加がみられた．

臓器重量はマウス，ラットに共通して体重抑制に対応した重量減少を示したが個々の臓器により多少の差はある．減少の最も目立ったものは雄マウスにおける肝，雄ラットにおける肝，睾，心，膵であった．

途中死亡例はマウスでは雄に多く，ラットでは雌に多かった．剖検所見では死亡例に一般的な諸器のうっ血以外に著変はなかった．組織学的にはマウス，ラット共に肝細胞の腫脹があり，マウスでは雄に，ラットでは雌に肝小葉中心部の肝細胞に軽度の脂肪沈着がみられた．

生存例の組織学的検索ではマウス 20, 40 mg/kg 群の半数例に肝細胞の変性と肝細胞の好酸性増加がみられた．ラットでは 64 mg/kg 群にのみマウスと同様の肝の変化と心筋の軽度変性，鼻の副鼻腔の増加，顎下腺終末細胞質の萎縮を示す例がみられた．これらの変化に薬物の一次的な作用によるものか，摂餌量の減少が原因となった二次的な作用かは判らないが，恐らく後者によるものと思われる．

ラットに6カ月間投与した場合は，30日間投与と略々同様の変化の他に雌にのみ GOT 値の増加，総 Cholesterol 値の減少がみられた．組織学的に病ちかな変化として，SD-JCL 系の老令雄ラットに一般的な変化即ち，脊 (Snell 1967) における管腔内にヒアリン様物質を満たし扁平な上皮からなる拡張した尿細管，上皮が好塩基性で空胞に富み一部肥厚した蛋白質を有する尿細管，炎症の細胞浸潤および軽度の線維化 ならびに膵 Langerhan 島 (Rana ら，1968, 緒下ら，1969) の線維化，ヘモジデリンの沈着，島の巨大腺等の変化は薬物投与群において明らかに減少した．これらの変化は摂餌量の減少に基づく二次的な変化か，薬物の直接の作用かは

Table 7 Hematological findings in rats

Test period	Dose (mg/kg/day)	No. of rats		Red blood cell ($\times 10^4$/mm³)		Hemoglobin (g/dl)	
		Male	Female	Male	Female	Male	Female
3 Months	0[a]	10	10	724	684	15.4	14.8
	2.5	10	10	683	681	15.3	14.9
	12.5	9	10	708	631*	15.0	14.5
	62.5	5	6	651*	584*	14.4*	13.1*
6 Months	0[a]	10	10	673	573	13.9	14.1
	2.5	10	10	704	591	15.3	14.5
	12.5	9	9	686	650	14.5	14.7
	62.5	3	6	660	647	15.3	14.4

a) 1% arabic gum.

Table 8 Organ weight of rats orally administered

Sex	Dose (mg/kg/day)	No. of rats	Heart (g)	Kidney (g)	Liver (g)	Spleen (g)	Absolute Adrenal (mg)
Male	0[c]	10	1.56	3.76	18.16	1.04	52.1
	2.5	10	1.44	2.88*	14.79*	0.85	54.0
	12.5	9	1.28*	2.67*	12.83*	0.60*	52.4
	62.5	3	0.98*	2.17*	10.55*	0.49*	49.7
Female	0[c]	10	1.00	2.00	10.49	0.64	66.5
	2.5	10	0.96	1.89	9.76	0.70	60.7
	12.5	9	0.88*	1.77*	9.02	0.55	54.4*
	62.5	5	0.88*	1.73*	8.27*	0.44*	62.0

a) Testis for male, and uterus for female.
b) Ventral prostate for male, and ovary (mg) for female.

Table 9 Plasma analysis of rats orally administered S-805 for 6 months

Sex	Dose (mg/kg/day)	No. of rats	Transaminase[a] S-GOT	S-GPT	Blood urea nitrogen (mg/dl)	Alkaline[a] phosphatase	Total cholesterol (mg/dl)	Glucose (mg/dl)	Total protein (g/dl)
Male	0[b]	10	40.5	7.8	17.3	18.4	124	188	6.4
	2.5	10	38.1	5.1	18.0	18.0	88*	177	6.6
	12.5	9	48.2*	9.6	17.2	20.5	74*	165*	6.6
	62.5	3	46.1	5.6	19.2	27.4	107	162	6.5
Female	0[b]	10	41.6	8.2	19.8	10.1	103	157	6.8
	2.5	10	39.8	6.3	18.0	12.5	85*	144*	6.5*
	12.5	9	50.9*	8.4	19.7	16.6*	81*	162	6.6
	62.5	5	70.5*	10.3	20.3*	14.6	82*	134*	6.4

a) Unit.
b) 1% arabic gum.
* Significant difference from controls, $p<0.05$.

igs in rats

orally administered S-805 for 6 months

Hematocrit (%)		White blood cell (×10³/cmm)		Differentiation (%) of WBC			
				Neutro.		Lympn.	
Male	Female	Male	Female	Male	Female	Male	Female
41.8	39.3	14,380	10,450	13.7	9.3	84.0	88.9
43.2	39.8	12,150	10,220	11.5	13.6	86.0	83.5*
41.6	39.2	10,800*	8,060*	13.9	21.5*	83.3	74.6*
39.6	36.5*	10,040*	10,150	22.2	32.5*	73.2*	64.7*
39.7	39.2	11,960	7,030	15.3	14.8	82.8	82.6
42.7	38.9	7,400*	6,950	12.4	19.1	85.2	77.1
42.9	40.7	10,460	7,956	17.8	19.9	80.2	78.4
42.3	35.2	8,600	7,440	19.3	27.6*	78.3	69.2*

* Significant difference from controls, $p<0.05$.

ninistered

S-805 for 6 months

Absolute

Organ Weight							
Thymus (mg)	Thyroid (mg)	Hypophysis (mg)	Submax. gl. (g)	Sex organ		Lung (g)	Cerebrum (g)
				(g)a)	(g)b)		
224.0	23.3	12.8	0.71	3.64	0.61	1.70	1.52
174.1	27.1	12.8	0.68	3.29	0.54	1.57	1.56
197.0	27.6	12.8	0.73	3.47	0.54	1.42*	1.54
131.0	24.4	10.8*	0.48*	3.10*	0.38*	1.26*	1.47
192.7	20.0	15.2	0.62	0.63	94.1	1.28	1.47
160.1	20.8	16.9	0.50	0.36*	97.4	1.26	1.47
147.0	18.5	13.4	0.50	0.31*	84.1	1.14	1.44
130.0	18.6	15.2	0.42*	0.38	67.6	1.13	1.44

a) 1% arabic gum

* Significant difference from controls, $p<0.05$.

Total protein (g/dl)
5.4
6.6
5.6
5.5
6.8
5.5*
6.6
6.4

se
d)

当らない.

ラットの亜急性と慢性毒性実験では最高投与量は時々ゆしいが死亡例はかなりの差があった. これは慢性毒性実験では実験の性格上動物の生涯の世界るだけ多くの前面に薬物を曝露させるため動物ラットでスタートしたことによる年令差による影響 (Zbinden 1963) と思われる.

本実験では一般に高い投与量を採用した. 即ちカタレプシーが約半数例にみられる 2.5 mg/kg を最小量として, 臨海作用を無視した大量を投与し, 現われるべき毒性の性格を知るための実験を行なった.

雄系は 62.5 mg/kg という大量 (LD_{50} の 1/2.5~1/3.6) 長期間投与によっても一部の例は死につながる強い薬理作用を示すが, 習慣的な障害は殆んどなく, 摂餌量の減少に基づく成長例制が最も著明な変化であった.

通常投与時にみられた変化の大部分は摂餌障害に起因した二次的な変化と思われる.

結　語

マウスおよびラットを用いて S-805 の 1 回および連続投与による毒性実験を行なった.

マウス, ラットに共通した薬物による一般状態の主な変化はカタレプシーと活動性の低下であり, 投与法, 投与回数, 性に関係なく認められた.

連続投与では摂餌量の減少, 成長抑制が投与量に比例してみられるが, マウス, ラット共に雄において, より著明であった. 成長抑制に対応して臓器重量の減少があり, 個々の臓器により減少の程度は異なるが雄マウスの肝, 雄ラットの肝, 腎, 肺, 心において明らかであった. 著明な重量増加を示した臓器はなかった.

薬物による死亡例はマウスでは雄に多く, ラットではその逆であった. 死亡例および 30 日間の大量投与例では肝細胞の壊死があり, その他に死亡例の雄マウスおよ

TABLE 10 Histological findings in rats orally administered S-805 for 6 months

Organ	Findings	Control[a] Male (10)[c]			Control[a] Female[b] (10)			2.5 mg/kg/day Male (10)			2.5 mg/kg/day Female (10)			12.5mg/kg/day Male (9)			12.5mg/kg/day Female (9)			62.5 mg/kg/day Male (3)			62.5 mg/kg/day Female (5)		
		++	+	±	++	+	±[d]	++	+	±	++	+	±	++	+	±	++	+	±	++	+	±	++	+	±
Liver	Atrophy of hepatic cell	0	1	1	0	3	0	1	1	3	0	1	3	0	1	1	0	1	3	1	0	1	1	1	2
	Decrease of cytoplasmic basophilia of hepatic cell	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0	2	2
	Periportal round cell infiltration	0	2	3	0	0	2	0	0	1	0	3	0	0	1	1	0	0	2	0	0	0	0	0	1
Kidney	Interstitial round cell infiltration	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Hyaline cast	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Tubular dilatation with flattened epithelium	2	2	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Heart	Infarct-like lesion	0	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pancreas	Fibrosis of islet	1	4	1	0	0	0	0	2	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Interstitial round cell infiltration	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
Lung	Perivascular hypercellularity	0	2	2	0	1	2	0	3	1	0	1	3	0	0	0	0	0	2	0	0	0	0	0	0
Spleen	Hemosiderosis	0	1	3	8	2	0	2	1	1	8	2	0	1	1	1	6	2	0	1	1	0	5	0	0
	Hyperemia	0	0	0	5	4	0	0	0	0	7	1	1	0	0	0	4	0	0	0	0	0	0	2	1
Bone marrow	Hypocellularity	0	0	0	0	2	0	0	2	0	1	2	0	0	0	0	0	3	3	1	0	1	0	2	1
Prostate	Interstitial round cell infiltration	0	0	0				0	0	1				0	0	1				0	1	0			
	Calculus	0	1	0				0	1	0				0	0	0				0	1	0			
Testis	Hypospermatogenesis	0	0	0				0	0	1				0	0	0				0	0	0			
Adrenal, Thyroid, Thymus, Stomach, Small intestine Large intestine, Mesenteric lymphnode, Cerebrum Ovary (Female), Uterus (Female)		Non-remarkable findings																							

a) 1% arabic gum

b) 1/10 mammary tumor (Fibroadenoma).

c) Figures in parentheses show numbers of rats observed.

d) A relative scale: (-) no lesion (omitted from the table), (±) slight, (+) moderate, (++) marked.

び雄ラットにのみ肝細胞に軽度の脂肪沈着がみられた.

ラットの6カ月間投与実験では組織学的に明らかな変化としてSD-JCL系老令雄ラットに一般的な腎および膵 Langerhans 島の自然発生の障害の減少が認められた.

文　献

Bliss, C.I. (1938): The determination of dose-mortality curve from small numbers. Quat. *J. Parm. and Pharmacol.*, **11**, 212–216.

城戸, 広瀬, 山本, 沢田, 小島, 永業 (1969) : 2-Chloro-11-(4-methyl-1-piperazinyl)-dibenzo [*b*, *f*] [1, 4] oxazepine (S-805) の中枢作用, 応用薬理, **3**, 361-372.

峰下, 村越, 矢原, 狗田, 上原, 川口, 岡田, 岡, 倉本 (1969) : マウスおよびラットにおける Cephalexin の毒性試験, Clinical Report, **3**, 390-416.

Rana, G., Hajdu, A. and Herr, F. (1968): Functional studies in rats with a spontaneous Langerhans islet alteration, *Amer. J. Pathol.*, **53**, 41a.

Snell, K.C.: Renal disease of the rat. In pathology of laboratory mice and rats edited by cotchin, E. and Roe, F.J.C. p. 105, Blackwell Scientific Publications (Oxford and Edinburgh), 1967.

Stille von G., Lauener, H., Eichenberger, Hunziker, F. und Schmitz, J. (1965): Uber ein neue chemische Gruppe starke wirksamer Neuroleptika. *Arzneimittelforschung*, **15**, 841–843.

Zbinden, G. (1963) : Experimental and clinical aspects of drug toxicity. *Advanc. Pharmac.* **2**, 183.

d) A relative scale: (−) no lesion (omitted from the table), (±) slight, (+) moderate, (#) marked.

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Special Report

TOXICITY TESTING OF 2-CHLORO-11-(4-METHYL-1-PIPERAZINYL)-DIBENZO [*b,f*] [1,4] OXAZEPINE (S-805) (FIRST REPORT)

Acute Toxicity, Sub-acute Toxicity and Chronic Toxicity Testing

Tetsuo Mineshita, Yoshihiro Muraoka, Isao Yahara, Tadayoshi Inuta, Michio Ishikawa, Junko Kawaguchi, Teruko Okada*

Toxicity Tests of 2-Chloro-11-(4-methyl-1-piperazinyl)-dibenzo [*b, f*] [1, 4] oxazepine (S-805) (I)

Acute, Subacute and Chronic Toxicity of S-805

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Toxicity studies of 2-chloro-11-(4-methyl-1-piperazinyl)-dibenzo [*b,f*] [1,4] oxazepine were performed in both sexes of the mouse and the rat. The studies consist of; 1) acute toxicity tests by a single dosage in mice and rats by the oral, subcutaneous, intraperitoneal and intravenous routes, 2) thirty days toxicity tests in mice and rats by the oral route at dose levels of 5,10,20 and 40 mg/kg/day for mice, and 0.5, 2, 8, 32 and 64 mg/kg/day for rats, 3) six months toxicity test in rats by the oral route at dose levels of 2.5, 12.5 and 62.5 mg/kg/day.

Sedation, ataxia, catalepsy, ptosis and respiratory depression were the main toxic signs which appeared in both mice and rats throughout the acute experiments. LD_{50} values by four routes of administration ranged from 22 to 76 mg/kg for mice and 18 to 381 mg/kg for rats. There were little differences in the values due the mode of administration in mice, but rats showed a large variation due to poor absorption of the drug by the subcutaneous route.

The animals showed hypoactivity, catalepsy and decrease of food intake with a dose-response relation in the continuous administration experiments. These changes caused growth retardation and subsequent decrease of organ weights.

Slight fat deposits in the hepatic cells at the central area of the lobules were found only in the animals which died during the treatment.

Spontaneous morphological changes in the kidney and the islets of the pancreas, which appeared in the aged males or the SD-JCL strain were apparently reduced in the drug treated groups in the six months experiment. It is unknown whether these findings were due to a primary effect of the drug or secondary to the decreased food intake.

(Received November 14, 1969)

INTRODUCTION

It has been thought that neuroleptics (major tranquilizers) can be chemically divided into a reserpine group, a benzoquinolizine group, a phenothiazine group, and a butyrophenone group, but recently, the activity of the benzoxazepine group as neuroleptics has come to garner attention (Stille, et al, 1965).

This time, we will report on our studies on the acute toxicity, sub-acute toxicity and chronic toxicity of the dibenzoxazepine derivative S-805 on mice and rats, as this is a compound which is highly anticipated as a new group of neuroleptics in relation to humans, and for which the conditional response inhibition activity, the spontaneous movement inhibition activity, the anti-apomorphine activity and the cataleptic activity has been confirmed in animal testing (Kido, et al, 1969).

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TEST MATERIAL AND METHOD

1. Compound

S-805 has the chemical structure shown in Figure 1, and as a white or yellow colored, odorless and tasteless crystalline powder, it is insoluble in water, but it is readily soluble in a wide variety of organic solvents including weak acids, and it has a melting point of 106~112°C (degradation).

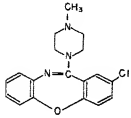

FIG 1 Chemical structure of S-805

2. Test method

Throughout this testing, we used ICR-JCL mice, and SD-JCL rats as the test subjects, and maintaining the animals in a subject room with a room temperature of 24~26°C and a humidity of 52~57%, we supplied the animals with Aburahi Laboratories solid food and tap water.

1) Acute toxicity

We administered the compound one time each orally, subcutaneously, intraperitoneally, and intravenously, to mice (20~29 g) and rats (130~200 g) which had fasted for 18~20 hours prior to administration of the compound, and dissolving a specific amount of the compound in 1N HCl, we adjusted the pH to 4~4.5 using bicarbonate soda prior to use. Using 4~9 dosages on both the mice and rats, with ten animals per dosage, the observation period was 3 days. Dissecting the fatalities and the subjects that managed to survive through the third day, we performed visual and invasive observations using H-E dyes.

We calculated the LD_{50} using the Bliss method (Bliss, 1938)

2) Sub-acute toxicity

For 30 days continuously, we orally administered S-805 that was suspended in a 1% gum Arabic to five groups of rats and four groups of mice, wherein there were twelve subjects per group. The dosage amount was 5, 10, 20, and 40 mg/kg per day for the mice, and for the rats, it was 0.5, 2, 8, 32, and 64 mg/kg. Using one group each as the control group, we administered only a 1% gum Arabic solution to these animals. During the dosage period, we performed measurements of the general state and weight of the animals as well as autopsies on the dead subjects, and we also measured the amount of food the subjects took. At the end of the testing, we randomly selected ten subjects from each group, and performing blood testing as well as dissection and measuring the weight of the internal organs, we performed a histological exam on ten rats and seven mice. When the number of surviving subjects within a group did not meet the above numbers, we performed this testing on all subjects within the group.

Please refer to the test results for the observations of the internal organs, and the details of the remaining observation methodology are as per a separate report (Mineshita, et al, 1969).

3) Chronic toxicity

The dosage amount was 2.5, 12.5, and 62.5 mg/kg per day. Using ten animals per group, we administered gum Arabic to the control group.

The dosage period was 6 months (26 weeks).

The observation items were selected as per the chronic test noted in the separate report (Mineshita, et al, 1969).

TEST RESULTS

1) Acute toxicity

Progression of the toxicosis: In all of the routes of administration (i.e. subcutaneously, intravenously, orally, or intraperitoneally) in both the mice and rats, we observed the same toxicity symptoms, a reduction in spontaneous movement, sedation, a slight relaxation in the skeletal muscles, difficulty walking and catalepsy, but in mice, there were subjects that showed convulsions, intoxication, and straub tail reactions prior to death. The onset of these symptoms was immediately after administration when administering the compound intravenously, it was 10 minutes later when administering the compound subcutaneously or intraperitoneally, and it was within 20 minutes when administering the compound orally. Within 30 minutes, we observed hypersalivation, lacrimation, respiratory inhibition, and drooping eyelids in addition to the above symptoms. These symptoms mostly disappeared within 48 hours, but the continuation of the catalepsy was long, taking up to 72 hours for the subject to return to normal. Also, at this point, the weight of the subject began to increase. Determinations of life or death for the animals were all made within 24 hours. Table 1 shows the LD_{50} values.

Visual and histological examination of the internal organs: In the dead subjects, we observed stasis in the liver, kidney, and lungs, but there were no other significant changes. In the mice that were put down on the third day,

TABLE 1 LD_{50} values of S-805 in mice and rats by single administration

Route	Mouse (mg/kg)		Rat (mg/kg)	
	Male	Female	Male	Female
po	67 (50~90)	62 (54~70)	221 (187~239)	151 (118~199)
sc	53 (44~62)	76 (70~82)	381 (331~439)	350 (292~409)
ip	34 (30~37)	34 (31~37)	35 (32~ 38)	37 (32~ 41)
iv	22 (20~24)	22 (20~24)	18 (18~ 19)	21 (19~ 23)

Figures in parentheses represent 95% confidence limits.

we observed only slight anemia in the lungs in those subjects that received an oral administration of the compound.

In the dead rats, we observed stasis of the liver, kidney, and lungs, and in the animals that were put down on the third day, we observed anemia in the liver, kidney, spleen, and thyroid gland. In the rat group that received subcutaneous administration of the compound, even in the animals that were put down on the third day, there were examples wherein a small amount of the compound was unabsorbed, and remained in the location of the compound administration.

There were no significant histological changes in the mice and rats.

2) Sub-acute toxicity

General state: In the mouse group that received the compound, throughout the entire test period, approximately 10 minutes after administration of the compound, we observed a relaxation of movement, a slight muscular relaxation, and catalepsy, and the strength and duration of the symptoms was related to the dosage amount. In rats, the 0.5 mg/kg group showed catalepsy and a slight sedative state only during the initial stage of the testing, in the 2 and 8 mg/kg groups, these symptoms lasted to the middle of the testing, and in the 32 and 64 mg/kg groups, these symptoms persisted throughout the entire period.

Fatalities: Table 2 shows the fatalities during the dosage period. In mice, the example which was clearly attributable to toxicity of the compound was only the 40 mg/kg group, and the fatality period was within 1 week of administration of the compound for all subjects. In the fatalities in this group, the weight of the subjects fell after the first day, and we observed a strong sedative state as well as catalepsy and hypersalivation. During the autopsy, we observed stasis of the liver, kidney, and lungs, as well as anemia and atrophy of the spleen. In the histological exam of these fatalities, we observed atrophy of the liver cells and a slight central fatty deposition in the male subjects, and in the female subjects, we observed only atrophy of the liver cells.

There were no other significant changes in the internal organs.

In rats, the male fatalities were only in the 64 mg/kg group, and we observed female fatalities in groups with dosages greater than 32 mg/kg, but in rats, unlike in the mice, there were many instances of fatalities among female subjects. Prior to death, we observed growth inhibition, and in particular, in the first half of the testing period, this growth inhibition was marked in the five subjects that died (45%). In the autopsy examination of the fatalities, we observed stasis of the liver, kidney, and lungs in all subjects, in half of the subjects, we observed atrophy of the spleen, and in a small number of the subjects, we observed nose bleeds and a bloating of the belly. Histologically, we observed a slight central fatty deposition of the liver cells in addition to the above stasis of the internal organs. This change was stronger in female subjects.

Food intake and body weight: In the mice, we observed body weight inhibition in the males of all of the groups that received the compound, and in females, we observed body weight inhibition in only the 40 mg/kg group, whereas the subjects in the other dosage groups showed a weight gain that was greater than or equal to that in the control group. The food intake showed the same trend as was seen in the body weight variation, and in the 20 mg/kg and lower dosages, the females showed a better eating efficiency than was even seen in the control. In the rats, there was growth inhibition in the dosage groups of 2 mg/kg and greater for the males, and 32 mg/kg and greater for the females, and we saw a correspondent reduction in the amount of food taken (Table 2).

Hemogram: There was no significant change in the red blood cell count in the mice. In the dosage groups of greater than or equal to 10 mg/kg, the males showed a trend in reduction of white blood cell count, but in the females, there was no variation with a dosage – activity relationship.

In rats, in the males in the 64 mg/kg group, there was a slight increase in the red blood cell count, the hemoglobin levels, and in the hematocrit values, and in this group, there was an increase in the white blood cell count in both the males and females. In terms of the white blood cell percentage by cell type, in the groups that received the compound, there was a general increase in heterophilic leucocytes, and we also observed a trend in reduction in the lymphocytes (Table 3).

Internal organ weight: In the mice, the internal organs that had seen a reduction in weight were the liver (males, greater than 10 mg/kg), thymus gland (males, 40 mg/kg), and adrenal gland (females, greater than 10 mg/kg), and the internal organ that saw an increase in weight was the submaxillary gland (females, greater than or equal to 20 mg/kg) (Table 4).

TABLE 2 Body weight and food consumption in mice and rats orally administered S-805 for 30 days

Species	Dose (mg/kg/day)	No. of animals		Average body weight gain (g)		Average food consumption (g/animal/30 days)		Food efficiency	
		Male	Female	Male	Female	Male	Female	Male	Female
Mouse	0[a]	12(0)[b]	12(1)	8.0	3.9	139	109	0.058	0.036
	5	12(1)	12(1)	4.6*	5.0*	142	107	0.032	0.047
	10	12(2)	12(0)	4.2*	5.0*	128	112	0.033	0.045
	20	12(1)	12(0)	3.7*	4.9*	127	129	0.029	0.038
	40	12(7)	12(4)	2.9*	3.3*	123	106	0.024	0.031
Rat	0[a]	12(0)	12(0)	260.1	120.3	749	530	0.347	0.227
	0.5	12(0)	12(0)	242.1	124.8	700	514	0.345	0.243
	2	12(0)	12(0)	226.2*	118.6	678	492	0.334	0.241
	8	12(0)	12(0)	196.4*	106.1	602	460	0.326	0.231
	32	12(0)	12(3)	157.0*	69.7*	533	401	0.295	0.174
	64	12(2)	12(6)	82.7*	35.7*	393	349	0.210	0.102

a) 1% arabic gum.

b) Figures in parentheses indicate the number of animals that died during the 30 days of administration.

* Significant difference from controls, $p<0.05$.

TABLE 3 Hematological findings in mice and rats

Species	Dose (mg/kg/day)	No. of animals		Red blood cell ($\times 10^4$/cmm)		Hemoglobin (g/dl)	
		Male	Female	Male	Female	Male	Female
Mouse	0[a]	10	9	883	909		
	5	10	10	833	893		
	10	10	10	842	892		
	20	10	10	935*	882*		
	40	5	8	924	868		
Rat	0[a]	10	10	721	730	12.6	13.9
	0.5	10	10	717	706	13.5	14.0
	2	10	10	719	743	13.2	13.9
	8	10	10	732	705	13.0	13.8
	32	10	9	718	651[b]	13.1	13.4
	64	10	6	764*	770	14.4*	14.6

a) 1% arabic gum.

TABLE 4 Organ weight in mice orally administered

Sex	Dose (mg/kg/day)	No. of mice	Heart (mg)	Kidney (g)	Absolute Liver (g)
Male	0[c]	10	165	0.60	2.06
	5	10	150	0.57	1.84
	10	10	176	0.63	1.72*
	20	10	167	0.59	1.55*
	40	5	177	0.62	1.68[b]
Female	0[c]	9	124	0.33	1.28
	5	10	129	0.35	1.35
	10	10	124	0.32	1.23
	20	10	136	0.34	1.28
	40	8	123	0.35	1.29

a) Seminal vesicle for male, and ovary for female.
b) Testis for male, and uterus for female.

In the male rats, at dosages of greater than or equal to 2 mg/kg, and in females, in the dosage groups of greater than or equal to 32 mg/kg, we observed a reduction in weight of each internal organ corresponding to the body weight reduction, and there were no internal organs which saw an increase in weight (Table 5).

Histological Exam: In the surviving mice, we observed a change attributable to the compound only in the male livers, and in the females, there was no change. The change in the liver was a slight increase in the acidophilic properties of the liver cells and a slight atrophy in the liver cells, which was confirmed in approximately half of the subjects in the 20 and 40 mg/kg groups.

We observed a slight atrophy in the liver cells of the surviving rats (5/10 subjects) and a reduction in the halophilic particles (3/10 subjects) in only the males in the 64 mg/kg group. The subjects that showed a slight atrophy in the heart muscles were the 64 mg/kg group (5/10 males, 2/6 females), and the 32 mg/kg group (2/10 males, 1/10 females). The subjects that showed an increase in the reticular cells surrounding the lymphoid follicles of the spleen and the subjects that showed a slight atrophy in the submaxillary gland end cells were found in the females in the 64 mg/kg group.

3) Chronic toxicity:

General state: in the 2.5 mg/kg group, during the early stages of the testing, we observed approximately half of the subjects showing a sedative state and symptoms similar to catalepsy, but after more than a month had passed, these subjects showed a general state that was in no way different from that of the control group. In the dosage groups of greater than or equal to 12.5 mg/kg, we observed the above symptoms in all of the subjects, and in particular, in the 62.5 mg/kg group, the above symptoms were strong, with many subjects dying within a week.

orally administered S-805 for 30 days

Hematocrit (%)		White blood cell (×1/cmm)		Differentiation (%) of WBC			
				Neutro.		Lymph.	
Male	Female	Male	Female	Male	Female	Male	Fema
		6,040	5,040	17.7	18.0	79.8	77.(
		5,800	3,360*	24.2	20.6	72.9	75.9
		3,820*	5,150	24.0	16.2	73.8	81.9
		3,200*	3,580*	24.6	14.0	73.3	82.(
		3,860*	4,890	23.0	13.4	75.0	83.(
38.5	39.8	8,530	7,120	7.5	7.3	90.9	90.:
38.1	38.7	8,870	7,340	8.4	11.7*	88.4	84.8
37.9	39.9	10,000	9,040	10.6	14.5*	86.1	82.8
37.5	39.0	7,800	9,170	15.3*	10.7	81.6*	87.1
37.7	34.7*	11,030	7,480	16.7*	15.0	80.4*	81.2
42.6*	39.2	12,220*	11,500*	11.4	12.7*	86.6	84.7

* Significant difference from controls, $p<0.05$.

S-805 for 30 days

organ weight

Spleen (mg)	Adrenal (mg)	Thymus (mg)	Submax. gl. (mg)	Sex organ (mg)[a)]	Sex organ (mg)[b)]	Lung (mg)
144	5.5	36.5	196	138	235	245
197*	6.1	30.1	170*	159	231	295
149	5.8	35.9	179*	185	227	233
124	5.5	31.7	207	207	226	233
122	5.8	25.4*	224	136	232	254
127	9.7	48.5	106	10.9	119	190
146	9.3	48.7	128	16.4*	122	201
135	7.4*	45.2	112	13.6	134	217
167	7.6*	56.5	135*	14.9*	151	226
130	7.9*	51.5	131*	13.8	132	201

c) 1% arabic gum.

* Significant difference from controls, $p<0.05$.

Fatalities: We observed a 64% mortality rate within the first week, and there was, in general, a strong growth inhibition prior to death. During an autopsy of these subjects, we observed stasis in the lungs, liver, and kidney, as well as bloating due to gas build-up in the digestive tract, and atrophy in the spleen. Histologically, our findings matched the fatalities in the sub-acute toxicity testing.
Body weight and food intake: In the males, there was a dosage – activity relationship in all of the dosage groups, but in the females, there was a growth inhibition in only the 62.5 mg/kg group, and we observed a reduction in the food intake corresponding to this inhibition (Table 6, Figure 2).
Hemogram: At the third month, in the 62.5 mg/kg group, we observed a trend for a slight reduction in red blood cell count, in the hemoglobin levels, and in the hematocrit levels, but at the sixth month, these values had returned to levels that were no different from those in the control group. The white blood cell count was slightly reduced in the third month in the males in the 12.5 and 62.5 mg/kg group. We observed an increase in the heterophilic leucocytes and a decrease in the lymphocytes in females in terms of the percentage of white blood count by cells, and we observed this as well in the 62.5 mg/kg group in the sixth month (Table 7).

Internal organ weight: Corresponding to the weight inhibition, the weight of the internal organs was, in general, reduced. While there is a significant difference in the degree of reduction in the various internal organs, this reduction was clear in the male liver, kidney, spleen, and heart (Table 8).
Blood plasma analysis: The changes with a dosage – activity relationship were in the S-GOT values of the females and in the total cholesterol levels. The former showed a higher value in the 12.5 and 62.5 mg/kg groups, and the latter showed a lower value in all dosage groups (Table 9).

TABLE 5 Organ weight of rats orally administered

Sex	Dose (mg/kg/day)	No. of rats	Heart (g)	Kidney (g)	Liver (g)	Absolute Spleen (g)
Male	0[c]	10	1.26	3.05	15.17	0.88
	0.5	10	1.19	2.85	14.54	0.93
	2	10	1.14	2.66*	13.42	0.79
	8	10	1.03*	2.36*	12.12*	0.76
	32	10	0.99	2.44*	10.67*	0.70*
	64	10	0.79*	1.88*	8.80*	0.45*
Female	0[c]	10	0.77	1.76	8.16	0.62
	0.5	10	0.85	1.83	7.50	0.68
	2	10	0.80	1.66	8.23	0.59
	8	10	0.76	1.70	7.96	0.61
	32	9	0.65	1.57	7.56	0.49
	64	6	0.57*	1.43*	6.91*	0.42*

a) Ventral prostate for male, and uterus for female.
b) Testis for male, and ovary (mg) for female.

TABLE 6 Body weight and food consumption in rats orally administered S-805 for 6 months

Dose (mg/kg/day)	No. of rats		Average body weight gain (g)		Average food consumption (g/animals/26 w)		Food efficiency	
	Male	Female	Male	Female	Male	Female	Male	Female
0[a]	10(0)[b]	10(0)	569	235	4,199	2,902	0.136	0.098
2.5	10(0)	10(0)	473*	280	3,629	2,724	0.130	0.103
12.5	10(1)	10(1)	401*	246	3,363	2,641	0.119	0.093
62.5	10(7)	10(5)	302*	197*	2,813	2,430	0.107	0.081

a) 1% arabic gum.
b) Figures in parentheses indicate numbers of rats that died during 6 months of administration.
* Significant difference from controls, $p<0.05$.

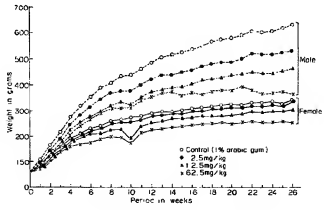

FIG 2 Growth curve of rats orally administered S-805 for 6 months.

S-805 for 30 days

organ weight

Adrenal (mg)	Thymus (mg)	Thyroid (mg)	Hypophysis (mg)	Submax. gl. (mg)	Sex organ (mg)a)	Sex organ (g)b)	Lung (g)
45.8	610	16.6	12.3	550	376	3.03	1.57
42.5	660	21.2	9.8*	530	340	3.08	1.95
52.0	510	19.0	11.9	530	361	3.16	1.47
52.5	470*	21.7	11.3	450	290	2.91	1.72
47.2	460*	13.3	9.5*	480	231*	2.66	2.13*
47.1	420*	12.7*	8.4*	390*	193*	2.54	0.98*
57.3	458	12.5	11.5	392	268	73.8	1.03
54.4	481	14.3	11.1	403	333	74.9	1.29
54.8	456	12.2	9.1	401	247	88.4	1.02
52.5	403	13.5	11.0	392	281	70.5	1.14
56.4	267*	11.5	8.9*	323*	214	53.5*	0.95
48.8*	260*	12.6	6.4*	273*	248	43.3*	1.04

c) 1% arabic gum.

* Significant difference from controls, $p<0.05$.

Histological exam: In males, we observed no significant obstruction due to the compound. However, the fibrosis of the pancreas islet and the dilation of the renal tubule due to the flattened lining that fills the hyaline column in the lumen, and the interstitial cell saturation in the kidney that is seen in elderly SD-JCL rats were clearly reduced in the groups that received the compound. In the females, we observed subjects in the 12.5 and 62.5 mg/kg groups that showed a reduction in the structure of the halophilic particles of the liver cells. There were no other significant changes relating to the administration of the compound (Table 10).

SUMMARY

Throughout the experiment, the general symptoms seen in both the mice and rats were the general catalepsy and sedative activity that is seen in tranquilizers, and these were observed with no relation to dosage method or gender. The strength of the activity and the duration was related to the dosage amount. In a single dosage, even when administering a lethal dose, those subjects that managed to survive were able to return to normal within 72 hours. The above activity is thought to be connected to the reduction in food intake during continuous administration of the compound as well as to the growth inhibition. With large doses that were administered continuously, the subjects died within one week as these symptoms increased, but the subjects that managed to survive during this period lived through to the end of the testing.

The reduction in food intake and growth inhibition were stronger in males than in females in both the mice and rats, and in the dosage group of less than or equal to 20 mg/kg, the females showed not only a food intake but also growth that was greater than that seen in the control groups.

There were no significant changes in the hemograms in the mice and rats, we observed a reduction in the white blood cell count in the dosage groups of 10 mg/kg and greater in the male mice. In the rats, there was an increase in the white blood cell count in both males and females in the 64 mg/kg group, we observed a slight increase in the red blood cell count in only the male subjects.

In terms of the weight of the internal organs, there was a weight reduction corresponding to the weight inhibition in both the mice and rats, but there was also a slight variation between the individual organs. The most significant cases were in the liver in male mice, and in the liver, kidney, heart, and spleen of the male rats.

There were many interim fatalities of male subjects in mice, and in rats, there were many female fatalities. In the autopsy exam, other than a general stasis of the internal organs, there were no significant changes. Histologically, there was atrophy in the liver cells in both the mice and rats, and with male mice and female rats, we observed a slight level of fatty deposition in the liver cells in the central part of the hepatic lobule.

In a histological exam of the surviving subjects, we observed an increase in acidophilic properties of the liver cells and atrophy in the liver cells in half of the subjects in the 20 and 40 mg/kg groups in mice. In the rats, we observed subjects showing atrophy in the submaxillary gland end cells, an increase in the reticular cells of the spleen, a slight atrophy of the heart muscle and similar liver changes as were seen in the mice, in only the 64 mg/kg group of rats. While it is unclear if these changes were due to a primary activity of the compound or if they were a secondary activity due to the reduction in food intake, but it is thought that it is probably due to the latter.

In the case of administering the compound to rats for 6 months, in addition to the same changes as were seen in the thirty-day administration, we observed an increase in GOT values and a reduction in total cholesterol levels in only the males. As a clear histological change, the general changes seen in older male SD-JCL rats, in other words, an enlarged renal tubule due to a flattened lining filling hyaline materials within the lumen in kidney (Snell, 1967), a renal tubule with a basal membrane that is partially thickened as the halophilic lining fills with aerocysts, fibrosis of the pancreas islet (Rana, et al, 1968; Mineshita, et al, 1969) and a slight fibrosis and interstitial cellular saturation, hemosiderin deposition, and enlargement of the islet, were clearly reduced in the groups receiving the compound. It is unclear whether these changes were secondary changes based on the reduction in food intake, or whether they were due to a direct activity of the compound.

TABLE 7 Hematological findings in rats

Test period	Dose (mg/kg/day)	No. of rats		Red blood cell ($\times 10^4$/cmm)		Hemoglobin (g/dl)	
		Male	Female	Male	Female	Male	Female
3 Months	0[a)]	10	10	724	684	15.4	14.8
	2.5	10	10	683	681	15.3	14.9
	12.5	9	10	703	631*	15.0	14.5
	62.5	5	6	661*	594*	14.4*	13.1*
6 Months	0[a)]	10	10	673	673	13.9	14.1
	2.5	10	10	704	691	15.3	14.5
	12.5	9	9	686	650	14.5	14.7
	62.5	3	6	660	647	15.3	14.4

a) 1% arabic gum.

TABLE 8 Organ weight of rats orally administered

Sex	Dose (mg/kg/day)	No. of rats	Heart (g)	Kidney (g)	Liver (g)	Spleen (g)	Absolute Adrenal (mg)
Male	0[c)]	10	1.56	3.76	18.16	1.04	52.1
	2.5	10	1.44	2.88*	14.79*	0.85	54.0
	12.5	9	1.28*	2.67*	12.83*	0.66*	52.4
	62.5	3	0.98*	2.17*	10.55*	0.49*	49.7
Female	0[c)]	10	1.00	2.00	10.49	0.64	66.5
	2.5	10	0.96	1.89	9.76	0.70	60.7
	12.5	9	0.83*	1.77*	9.02	0.55	54.4*
	62.5	5	0.88*	1.73*	8.27*	0.44*	62.0

a) Testis for male, and uterus for female.
b) Ventral prostate for male, and ovary (mg) for female.

TABLE 9 Plasma analysis of rats orally administered S-805 for 6 months

Sex	Dose (mg/kg/day)	No. of rats	Transaminase[a)] S-GOT	S-GPT	Blood urea nitrogen (mg/dl)	Alkaline[a)] phosphatase	Total cholesterol (mg/dl)	Glucose (mg/dl)	Total protein (g/dl)
Male	0[b)]	10	40.6	7.8	17.3	18.4	124	188	6.4
	2.5	10	38.1	5.1	18.0	18.0	88*	177	6.6
	12.5	9	48.2*	9.6	17.2	20.5	74*	165*	6.6
	62.5	3	46.1	5.6	19.2	27.4	107	162	6.5
Female	0[b)]	10	41.6	8.2	19.8	10.1	103	157	6.8
	2.5	10	39.8	6.3	18.0	12.5	85*	144*	6.5*
	12.5	9	50.9*	8.4	19.7	16.6*	81*	162	6.6
	62.5	5	70.5*	10.3	20.3*	14.6	82*	134*	6.4

a) Unit. b) 1% arabic gum.
* Significant difference from controls, $p<0.05$.

orally administered S-805 for 6 months

Hematocrit (%)		White blood cell (×1/cmm)		Differentiation (%) of WBC			
				Neutro.		Lymph.	
Male	Female	Male	Female	Male	Female	Male	Female
41.8	39.3	14,380	10,430	13.7	9.3	84.0	88.9
43.2	39.8	12,150	10,220	11.5	13.6	86.0	83.5*
41.6	39.2	10,300*	8,090*	13.9	21.8*	83.3	74.6*
39.6	36.5*	10,040*	10,150	22.2	32.5*	73.2*	64.7*
39.7	39.2	11,950	7,030	15.3	14.3	82.8	82.6
42.7	38.9	7,400*	6,950	12.4	19.1	85.2	77.1
42.9	40.7	10,400	7,956	17.8	19.9	80.2	76.4
42.3	35.2	8,600	7,440	19.3	27.6*	78.3	69.2*

* Significant difference from controls, $p<0.05$.

S-805 for 6 months

Organ Weight							
Thymus (mg)	Thyroid (mg)	Hypophysis (mg)	Submax. gl. (g)	Sex organ		Lung (g)	Cerebrum (g)
				(g)[a)]	(g)[b)]		
224.0	28.3	12.8	0.71	3.64	0.61	1.70	1.52
174.1	27.1	12.8	0.68	3.29	0.54	1.57	1.56
197.0	27.6	12.8	0.73	3.47	0.54	1.40*	1.54
131.0	24.4	10.8*	0.48*	3.10*	0.38*	1.25*	1.47
192.7	20.0	15.2	0.52	0.63	94.1	1.28	1.47
160.1	20.8	16.9	0.50	0.36*	97.4	1.26	1.47
147.0	18.5	13.4	0.50	0.31*	84.1	1.14	1.44
130.0	18.6	16.2	0.42*	0.38	67.6	1.13	1.44

c) 1% arabic gum.

* Significant difference from controls, $p<0.05$.

In the rat sub-acute and chronic toxicity testing, while the maximum dosages administered were approximately equal, there was a significant difference in the fatality rate. This is thought to be the effect of age differences as we started with juvenile rats in order to expose the compound on as many cross-sections as possible during the life of the above animals in the chronic toxicity testing (Zbinden, 1963).

In the present testing, we used a generally high dosage. In other words, using 2.5 mg/kg as the minimum dosage, for which we observed catalepsy in approximately half of the subjects, we administered a large dosage regardless of the pharmacological activity, and performed this testing in order to determine the toxicity characteristics that would arise.

The results showed that even with a large dose such as 62.5 mg/kg (1/2.5~1/3.6 LD_{50}) that was administered over a long period of time, there was a strong pharmacological activity that led to death in a portion of the subjects, but there was little or no organic obstruction, and the most significant change was the growth inhibition based on the reduction in food intake.

A large part of the changes seen during continuous dosage were thought to be secondary changes due to a reduction in the food intake.

CONCLUSIONS

Using mice and rats, we performed toxicity testing through single administration and continuous administration of S-805.

In both the mice and rats, the main changes to the general state of the animals due to the compound were a reduction in activity and catalepsy, and these were seen with no relationship between dosage method, dosage frequency or gender.

During continuous dosage, the reduction in food intake and the growth inhibition was correlated to the dosage amount, but these were more pronounced in males in both the mice and rats. There was a reduction in the weight of the internal organs corresponding to the growth inhibition, and while the level of reduction was different in the individual organs, it was clear in the male mice livers, and in the liver, kidney, spleen, and heart of male rats.

Fatalities due to this compound were frequent in mice, and the reverse was true in rats. In the subjects receiving a large dosage for 30 days and in the fatalities, we observed atrophy in the liver cells, and we observed a slight level of fatty deposition in the liver cells in only female rats and male mice that had died.

TABLE 10 Histological findings in rats orally administered S-805 for 6 months

Organ	Findings	Control[a]						2.5 mg/kg/day						12.5mg/kg/day						62.5 mg/kg/day					
		Male (10)[c]			Female[b] (10)			Male (10)			Female (10)			Male (9)			Female (9)			Male (3)			Female (5)		
		#	+	±	#	+	±[d]	#	+	±	#	+	±	#	+	±	#	+	±	#	+	±	#	+	±
Liver	Atrophy of hepatic cell	0	1	1	0	3	0	1	1	3	0	1	3	0	1	1	0	1	3	1	0	1	1	1	2
	Decrease of cytoplasmic basophilia of hepatic cell	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0	2	2
	Periportal round cell infiltration	0	2	3	0	0	2	0	0	1	0	3	0	0	1	1	0	0	2	0	0	0	0	0	1
Kidney	Interstitial round cell infiltration	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Hyaline cast	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Tubular dilatation with flattened epithelium	2	2	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Heart	Infarct-like lesion	0	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pancreas	Fibrosis of islet	1	4	1	0	0	0	0	2	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Interstitial round cell infiltration	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
Lung	Perivascular hypercellularity	0	2	2	0	1	2	0	3	1	0	1	3	0	0	0	0	0	2	0	0	0	0	0	0
Spleen	Hemosiderosis	0	1	3	8	2	0	2	1	1	8	2	0	1	1	1	6	2	0	1	1	0	5	0	0
	Hyperemia	0	0	0	5	4	0	0	0	0	7	1	1	0	0	0	4	0	0	0	0	0	0	2	1
Bone marrow	Hypocellularity	0	0	0	0	2	0	0	2	0	1	2	0	0	0	0	0	3	3	1	0	1	0	2	1
Prostate	Interstitial round cell infiltration	0	0	0				0	0	0				0	0	1				0	1	0			
	Calculus	0	1	0				0	1	0				0	0	0				0	1	0			
Testis	Hypospermatogenesis	0	0	0				0	0	1				0	0	0				0	0	0			

Adrenal, Thyroid, Thymus, Stomach, Small intestine
Large intestine, Mesenteric lymphnode, Cerebrum
Ovary (Female), Uterus (Female)

Non-remarkable findings

a) 1% arabic gum.
b) 1/10 mammary tumor (Fibroadenoma).
c) Figures in parentheses show numbers of rats observed.
d) A relative scale: (−) no lesion (omitted from the table), (±) slight, (+) moderate, (#) marked.

In the six-month dosage testing in rats, we confirmed that a general reduction in the naturally occurring obstruction of the pancreas islet and kidney in older SD-JCL rats was the clear histological change.

CITED LITERATURE

Bliss, C.I. (1938): The determination of dose-mortality curve from small numbers. Quat. *J. Parm. and Pharmacol.*, **11**, 212–216.

Kido, Hirose, Yamamoto, Sawada, Kojima, Eigyo (1969): Central Nervous System Activity of 2-Chloro-11-(4-methyl-1-piperazinyl)-dibenzo [*b,f*] [1,4] oxazepine (S-805), Applied Pharmacology, 3, 361-372.

Mineshita, Muraoka, Yahara, Inuta, Kawahara, Kawaguchi, Okada, Oka, Kuramoto (1969): Toxicity Testing of Cephalexin in Mice and Rats; Clinical Report, 3, 390-416.

Rana, G., Hajdu, A. and Herr, F. (1968): Functional studies in rats with a spontaneous Langerhans islet alteration, *Amer. J. Pathol.*, **53**, 41a.

Snell, K.C.: Renal disease of the rat. In pathology of laboratory mice and rats edited by cotchin, E. and Roe, F.J.C. p. 105, Blackwell Scientific Publications (Oxford and Edinburgh), 1967.

Stille von G., Lauener, H., Eichenberger, Hunziker, F. und Schmitz, J. (1965): Uber ein neue chemische Gruppe starke wirksamer Neuroleptika. *Arzneimittelforschung*, **15**, 841–843.

Zbinden, G. (1963) : Experimental and clinical aspects of drug toxicity. *Advanc. Pharmac.* **2**, 183.